PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	19. 11. 99
Applicant's or agent's file reference VIB-08-WM/M2	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/05106	05/08/1998	05/08/1997

Applicant
VLAAMS INTERUNIVERSITAIR INSTITUUT ....

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
VIB-08-WM/M2		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/05106	05/08/1998	05/08/1997
International Patent Classification (IPC) or national classification and IPC		
C12N15/00		
Applicant		
VLAAMS INTERUNIVERSITAIR INSTITUUT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/03/1999	19. 11. 99
Name and mailing address of the international preliminary examining authority:	Authorized officer
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Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/05106

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

D scription, pages:

1-59	as originally filed				

Claims, No.:

1-25	as received on	10/11/1999	with letter of	09/11/1999	

Drawings, sheets:

1/35-35/35	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. R asoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-25
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-25
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations

see separate sheet

V. REASONED STATEMENT UNDER ARTICLE 35(2)

1) The present application relates to the generation of an antigen for eliciting a protective immune response against influenza infection. Said antigen comprises an immunogenic part of an influenza membrane protein and a presenting carrier, usually a polypeptide or alternatively a glycan, polyethylene, or others. The presenting carrier is included in the antigen in order to facilitate the preservation of the wild type structure of the immunogenic polypeptide and hence, potentiate the immunogenicity of said fusion antigen. The fusion antigen is shown to impart protection against influenza infection, and improved antigenicity compared to known M2 polypeptide antigens.

2) The subject-matter of **Claim 1** relates to an influenza fusion antigen with the following technical features:
(i) comprising at least an extracellular part of the M2 influenza membrane protein or a functional fragment thereof
(ii) comprising a presenting carrier
(iii) capable of eliciting a statistically significant higher immunoprotection when administered to animals compared to animals not receiving said fusion antigen.

According to the description of the present application, recombinant M2 protein produced in a baculovirus system was used as antigen in a vaccine and induced protective immunity in immunized mice (see also document (D2) Vaccine, 1995, vol. 13, pages 1399-1402, submitted by the Applicant). Furthermore, the protective capacity of antibodies directed against the extracellular part of the M2 integral membrane protein of the influenza A virus was demonstrated when said antibodies were administered to animals (p. 5 of the present application).

The problem to be solved appears to be the provision of an antigen derived from the influenza M2 protein with improved antigenicity.

The Applicant suggested the generation of a fusion antigen comprising the M2 protein extracellular part and a presenting carrier. The suggestion solves the problem.

Document (D1): AU 49273 90 A, 9 August 1990, discloses that in order to improve the presentation of a particular viral epitope (FMDV, foot and mouth disease virus), a fusion polypeptide is generated that comprises said epitope and the polypeptide of the viral coded core antigen (HBcAg). The same document indicates the generation of fusion antigens comprising a viral epitope of the influenza virus. Document D2 , on the other hand, indicates a number of epitopes present in the M2 protein which elicit an immune response (Table 2) and even more specifically, it discloses that an epitope in the C-terminal cytoplasmic domain of the M2 protein elicited a substantial immune response even greater than that of the epitope originating from the N-terminal region of the protein (Table 2 and p. 1401). Therefore, the skilled person faced with the above mentioned problem following the teachings of document D2 would have a number of epitopes to choose from in order to generate a fusion protein according to the teachings of document D1. There is no indication in either document guiding the skilled into using the extracellular part of the M2 protein to generate a fusion protein to be used as an improved antigen.

Therefore, the subject-matter of **Claims 1-25** is novel and inventive as required by Article 33(2) and (3) PCT.

CLAIMS

1. An influenza antigen comprising a fusion product of (i) at least an extracellular part of an influenza M2 membrane protein or a, functional fragment thereof or modified versions thereof and (ii) a presenting carrier, wherein said extracellular part contains all or part of the 23 amino acids extracellular domain (amino acids 2 to 24 as shown in Table 1) of an M2 protein of influenza A virus or of similar integral membrane proteins of influenza B and C, and wherein said functional fragment is a fragment of an M2 protein capable of eliciting a statistically significant higher immunoprotection when administered in an immunoprotective dose to test members of a species compared to test members of the same species not receiving the functional fragment, and wherein said modified versions comprise one to three amino acid changes but still react with a polyclonal antiserum derived from immunized mice.
2. The influenza antigen as claimed in claim 1, wherein the presenting carrier is a presenting (poly)peptide.
3. The influenza antigen as claimed in claim 1 or 2, wherein the presenting carrier is a non-peptidic structure, such as glycans, peptide mimetics, synthetic polymers.
4. The influenza antigen as claimed in any of claims 1 to 3 further comprising an additional domain for enhancing the cellular immune response immunogenicity of the antigen.
5. The influenza antigen as claimed in any of claims 1 to 4, wherein the presenting (poly)peptide is selected from the hepatitis B core protein, one or more C3d domains and tetanus toxin fragment C.
6. The influenza antigen as claimed in any of claims 1 to 5, wherein said presenting carrier not substantially alters the tertiary structure of said part of the protein
7. The influenza antigen as claimed in any of claims 1 to 6, wherein the antigen consists of Lactococci cells expressing the fusion product in or on their cell membrane, optionally said cells release said product.
8. The influenza antigen as claimed in claim 4, wherein the additional domain is an influenza specific T helper cell epitope or cytotoxic T cell epitope.
9. A gene construct encoding an antigen as defined in any of claims 1 to 8.

10. The influenza antigen as claimed in any of claims 1 to 8, obtainable by preparing a gene construct according to claim 9, comprising a first coding sequence encoding an extracellular part of an influenza membrane protein or a functional fragment thereof or modified versions thereof as defined in claim 1, and at least one second coding sequence for a presenting (poly)peptide operable linked thereto, optionally in the presence of suitable transcription and/or translation regulatory sequences.

11. The influenza antigen as claimed in any of claims 1 to 8 or 10 for use in the preparation of a vaccine against influenza for humans and/or animals.

12. The influenza antigen as claimed in any of claims 1 to 8 and 10 for use in the preparation of a vaccine against influenza A for humans and/or animals.

13. A vaccine against influenza, comprising at least an antigen as claimed in any of claims 1 to 8 and 10, optionally in the presence of one or more excipients.

14. The vaccine as claimed in claim 13, wherein the antigen is in isolated form.

15. The vaccine as claimed in claim 13, wherein the antigen is part of a membrane fragment.

16. The vaccine as claimed in claim 13, wherein the antigen is anchored in the membrane of an acceptor cell expressing the antigen.

17. The vaccine as claimed in claim 13, wherein the antigen consists of Lactococci cells expressing the fusion product in or on their cell envelope.

18. The vaccine as claimed in any of claims 13 to 17, further comprising one or more other influenza antigens, for example selected from hemagglutinin, neuraminidase nucleoprotein and/or native M2.

19. A DNA vaccine comprising a gene construct according to claim 9.

20. A vaccinia based vaccine comprising a gene construct according to claim 9.

21. Use of an antigen as claimed in any of claims 1 to 8 or 10 to 12 for the preparation of a vaccine against influenza.

22. Use of a gene construct as claimed in claim 9 for the preparation of a DNA based or a vaccinia based vaccine against influenza.

23. Method of preparing an antigen as claimed in any of claims 1 to 8 or 10 to 12, comprising the st ps of:

    a) preparing a gene construct according to claim 9,

AMENDED SHEET

b) bringing this gene construct in a suitable acceptor cell,

c) effecting expression of the gene construct in the acceptor cell, and

d) optionally isolating the antigen from the acceptor cell or its culture medium.

24. An acceptor cell, expressing an antigen as claimed in any of claims 1 to 8 and 10 to 12.

25. The acceptor cell as claimed in claim 24, wherein the cells are Lactococcus cells.